Issued February 3, 1908.

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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF CHEMISTRY—BULLETIN No. 111.

H. W. WILEY, Chief of Bureau.

THE FERMENTING POWER OF PURE YEASTS AND SOME ASSOCIATED FUNGI.

By

WM. B. ALWOOD,

IN CHARGE, ENOLOGICAL-CHEMICAL INVESTIGATIONS.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., December 9, 1907.

Sir: I have the honor to submit for your approval a paper on the fermenting power of pure yeasts and some associated fungi, prepared by Mr. Wm. B. Alwood, in charge of the enological-chemical investigations making in this Bureau. This report is a continuation of the investigations already reported in Bulletins 71 and 88 of the Bureau of Chemistry, and deals with fundamental facts of importance in the manufacture of by-products from fruits, which work it is our purpose to extend, with a view to improving the quality and purity of the products, by the introduction of better technique and the application of the results of scientific investigation. I recommend that the report be published as Bulletin 111 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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INTRODUCTION.

The use of selected pure yeast cultures has become a common practice in certain industries, notably in brewing and distilling, and their value in other lines, as in cider manufacture and in bread making, is now recognized. Selected, pure races of yeasts have come into much more general use in the cider industry in Germany than in other countries, and when employed with the proper technique they have been found very beneficial. Their use in this industry has been practiced to a considerable extent for some years past in France, and less extensively in this country, yet there is every reason to believe that the industry would be greatly benefited by their employment.

As special agent of the Bureau of Chemistry the writer collected from foreign laboratories a number of tested yeasts and has also isolated a considerable number of yeasts from native and foreign sources. These have been tested in the fermenting of apple juice for several years past, incidentally in connection with other investigations, and pure yeast cultures have also been distributed quite widely in the United States for the past six years, some of which seem to be valuable for cider making. A description of these yeasts is given on page 25.

There is, however, a very large amount of work necessary on the practical details, as well as more critical scientific investigation, before pure yeast cultures can be sent to local manufacturers with a fully developed plan for their use in ordinary factory work. In fact, the question greatly needs at this time the assistance of well-equipped cider manufacturers in working out many details, and to those who wish to experiment with special yeast cultures these will be furnished, together with such data and instructions as are obtained in the experimental work.

TESTING PURE YEASTS.

The fact that a yeast is a pure culture does not settle the question of its value for cider making, hence when an apparently desirable

pure yeast has been isolated it is necessary to subject it to scientific and practical tests. The measurement of the yeast's activity as a chemical reagent in breaking down sugars in solution has been the scientific test used. This test is so devised as to obtain a direct time measure of the fermenting power of the yeast and to determine the products resulting from its growth in the liquor fermented. Only those yeasts which have been isolated in accordance with correct technique and have shown good characteristics, such as vigor, brightness of resultant liquor, etc., and thus warrant further investigation, are subjected to the chemical test in culture flasks.

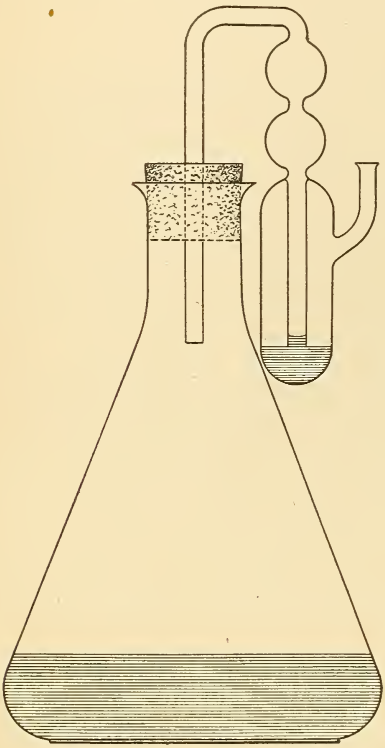

Fig. 1.—Culture flask for fermentation tests.

CULTURE FLASK.

This test is accomplished by sowing the yeast cultures into flasks so prepared that the growth activities of the yeast may be observed, the loss of carbon dioxid estimated, and, after fermentation is completed, the resultant products determined by analysis without complication of the results by the growth of other organisms. A flask prepared for this work is shown in fig. 1. It consists ordinarily of a stout, clear bottle, or of an Erlenmeyer flask of 800 cc capacity. In this flask are placed 400 cc of a filtered must or other prepared culture medium, the composition of which has been determined by analysis. The flask or bottle is then stoppered with a perforated rubber stopper, through which is fitted the ventilating tube, shown in fig. 2. The stem of the tube, inserted through the stopper, should not extend down near the liquor, as when fermentation is active the slightest agitation will tend to throw the foam through the ventilating tube and the test will be lost. The outer end of the tube is curved down onto the neck of the bottle or flask and carries a control apparatus consisting of a flask-like enlargement of the tube, with a small inner tube which dips down into the control liquid contained in the enlarged outer portion of the ventilating

apparatus. A small funnel-shaped opening permits the gas to escape during fermentation.

This ventilating apparatus not only permits the gas to escape, but makes it possible to observe the activities going on within the flask and also protects the medium from contamination by air. The growth of the yeast is noted by the rate of passage of gas through the control liquid, which should be 5 to 10 per cent sulphuric acid. This device, when the control liquid is in place, makes it impossible for germs to enter if, by change of temperature, air should be drawn into the bottle or flask. It also renders evaporation of the culture medium impossible to any appreciable degree during the short period of the test.

The stopper carrying the control apparatus is fixed in the bottle or flask after the measured, or preferably weighed, quantity of culture medium has been placed in it, and the neck of the bottle or flask, including the cork, is closely wrapped with parchment paper held in place by a rubber band. The outer funnel opening is plugged with cotton. The whole apparatus, with its contents, is then sterilized by steam heat and set away, ready to be sown with the organism to be tested. For acid fruit juice one sterilization, heating for forty minutes in steam at 100° C., is sufficient, but for malt or like substances this should be repeated on two successive days.

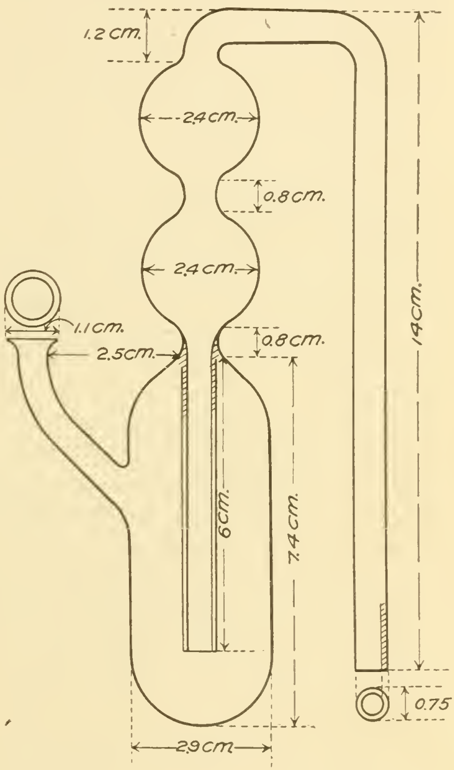

Fig. 2.—Detail of ventilation tube for culture flask.

When making this test, it is of the greatest importance to sow under conditions which, as far as possible, prevent the entrance of extraneous organisms. It is also important to sow all cultures with a standard loop of platinum wire prepared to carry a drop of uniform size. Otherwise, tests made at different times in the laboratory may show a different rate of fermentation at inception, not by reason of greater or less activity on the part of the yeast used, but because of the greater

or smaller quantity of the organism introduced into the bottle or flask. Also it is equally important to sow only from cultures in active growth, otherwise the promptness with which fermentation follows may be very much influenced and unreliable results obtained. Ordinarily, yeast cultures in 10 cc of liquid culture medium in test tubes will be ready to use in three days and will remain strong for ten days. While the yeast in these tube cultures remains alive for many months, cultures from three to ten days old are necessary for the tests under consideration.

When all is ready for the test of the chemical activity of the yeast, the bottle or flask is sown with a drop of the fluid culture taken direct from a tube in fermentation. The flask is then carefully closed and waxed over the stopper about the opening, so as to prevent the entrance or escape of air or gas except through the control apparatus. For this purpose warm paraffin wax is very serviceable and is cleaner to handle than prepared wax. Ordinarily, a number of organisms will be sown in separate flasks to be tested at the same time, or the same organism may be tested in various musts and artificial saccharine solutions. The flasks designated for comparison should all be kept together in a culture oven at a temperature varying from 20° to 25° C., as this is thought to be the best range of temperature for yeast organisms.

Each flask should be labeled as sown. A record of the yeast sown and the medium used in each bottle is kept for identification. Each bottle or flask is weighed as soon as sown, then once each day at the same hour each flask is reweighed and these data are entered in the records. The form of record prepared for use in this laboratory (see p. 11) gives the history of the test and the daily weights, the daily loss caused by the escape of gas from the fermenting substance, and the temperature readings, together with analyses, etc. Such a record comprises the biological and chemical data of the test, and, in connection with the critical notes giving the appearance of the liquor, the characteristics of the foam and of the bouquet or odor, taken as the gas escapes, the subsidence of the deposit, and other memoranda, furnishes a basis for judging of the value of a yeast. Upon these data, if obtained under proper conditions, one may base a conclusion as to whether any particular yeast promises to be valuable for practical work. Such tests do not, however, settle the question. In fact, the value of a yeast for vinous fermentation is not conclusively established until it has been used in normal must, in regular cellar work, on a sufficiently large scale to give practical results. This chemical test is most essential, however, and should precede the practical test if definite results are to be obtained.

Many yeasts produce quite similar amounts of alcohol when sown in the same must and leave liquors when fermentation is complete,

which, judged only by analytical data, reveal only slight individual differences. On the other hand, there are some well-tested yeasts which show a considerable difference in the amount of alcohol produced from the fruit juice. Also, in certain cases, the finished products from the same must, fermented by different yeasts, has been pronounced quite dissimilar when judged by experts. These dissimilarities in the products derived from the same fruit juice, when fermented by different pure yeasts under like conditions, must result from peculiar vital qualities of the particular yeast organism which dominates the fermentation. The chemical composition and peculiar qualities developed in cider by the use of some of the pure races of yeasts selected and tested by the writer have been described in a previous report.[a] No attempt is there made to explain or discuss the peculiar qualities, seemingly inherent in the yeasts, upon which the results obtained seem to depend. The data are as yet insufficient to warrant a critical discussion of these phenomena, yet the results thus far obtained are so illustrative of the chemical activities of these organisms that some examples are presented as a contribution to the study of the yeast ferments.

The work reported in the study of the composition of ciders as determined by dominant fermentation with pure yeasts (Bulletin 88) was carried on in 50-gallon casks, thus approaching commercial conditions. Such experiments, unfortunately, do not afford sufficient opportunity for the intimate study of the individual peculiarities of the yeast, and the yeasts used in the practical experiments reported in the bulletin mentioned were first subjected to the laboratory test in fermentation flasks.

Later and more detailed fermentation tests in the laboratory have included a considerable number of yeasts and some associated fungi common to fruit musts. Only such of the tests are here reported as show possibilities for future development and indicate the direction for further studies.

RECORD SHEET.

The record sheet devised for use in the study of the chemical activity of yeasts in this laboratory contains the following data, which are arranged in tabular form on one sheet for filing:

Date, October 24, 1902. *TEST NO.* 68. *Yeast No.* 8.

Sowed at 2 p. m., *400 cc. Must*, Apple. *No.* 4. *Vessel*, Erlenmeyer, *with organism from Tube No.* 8. *Sown (date)* Oct. 24, 1902.

[a] U. S. Dept. of Agr., Bureau of Chemistry, Bul. 88, the Chemical Composition of Apples and Cider.

Weight at (hr.), 3.30 p. m., 633.92 *grams.*

Purpose of test, comparison of pure yeast races of French, German, and American origin with each other and in mixed sowing.

Conducted by * * *

Yeast growth observed at 9 a. m. Oct. 26, *1902.*
First gas noted, 9 a. m. Oct. 26, *1902.*
Total hours of experiment, 427.
Total hours before fermentation, ----.
Total hours duration of fermentation, 408.
Total loss in grams, 26.30.
Average temperature, 25.5° C.
Range of temperature, 5.0° C.
Sent to chemist, Nov. 11, 1902.
Analysis reported, ----.

Daily observations.

Date.	Weight at 9 a.m.	Loss.	Temperature. 9 a. m.	Temperature. 4 p. m.
Oct. 25	633.90	0.02	24	25
26	633.72	.18	20	22.5
27	628.87	4.85	23	23
28	624.02	4.85	23	23
29	619.92	4.10	22	22.5
30	616.60	3.32	22	23
31	613.90	2.70	21	22.5
Nov. 1	611.60	2.30	22.5	23.5
2	609.94	1.66	23	23
3	609.02	.92	22.5	22.5
4	608.60	.42	21.5	22
5	608.32	.28	22.5	24.5
6	608.12	.20	21	23.5
7	607.95	.17	23	23
8	607.80	.15	21.5	22.5
9	607.70	.10	20	21
10	607.67	.03	20	23.5
11	607.60	.07	24	----------

Analytical data by * * *

(Results are given in grams per 100 cc.)

Original must.	Determinations.	Fermented liquor.
1.066	Specific gravity	1.003
----------	Alcohol	5.02
13.15	Sugar, total	.15
10.36	Sugar, reducing	.15
2.65	Sucrose	----------
14.60	Solids, total	2.78
.51	Fixed acid, as H_2SO_4	.55
----------	Acid, volatile, as acetic	.02
----------	Acid, total, as	----------
.018	Tannin	.016
----------	Glycerol	----------
----------	Ash	----------

(Note sheets are attached for record of general observations.)

DISCUSSION OF THE TESTS.

Some of the experiments conducted during the past four years seem to be of a striking significance in connection with the development of the fermentation industries. It appears that the question of

the use of pure yeasts for securing dominant fermentation in unsterilized must is of great importance in the manufacture of beverages and vinegar from fruit juices. The difficulties which constantly arise in the fermentation rooms or in the vinegar factory are many, and any practicable method of insuring a strong, active fermentation which will overcome the various malferments that cause trouble and disease in the fermenting must will be valuable. The experiments previously made and the analyses of the products obtained clearly showed the value of the use of pure yeasts in fresh fruit juices. The following tests, with small amounts of apple juice sown with pure yeasts in flasks of sterilized and unsterilized must, further illustrate this point.

GROWTH OF YEAST IN STERILIZED AND UNSTERILIZED JUICE.

Four flasks, each containing 400 cc of fresh juice, were used for this test. No. 1 was left unsown as it came from the press. No. 2 was sown when fresh from the press with 1 cc of a vigorous culture of yeast No. 8. Nos. 3 and 4 were quickly sterilized by holding at steam heat for twenty minutes in a sterilizer, then rapidly cooled under the water tap and sown with yeasts, as follows: No. 3 received 1 cc of yeast No. 8 and No. 4, 1 cc of yeast No. 66. These four flasks were then put in the culture oven and kept at a temperature ranging from 21° to 27° C. for a period of twenty-one days, at which time the weights became nearly constant. The following tabular statement shows the composition of the original juice and of each flask at the close of the period.

TABLE I.—*Composition of original must and of four flasks differently treated, after a period of twenty-one days (analysis by Bureau of Chemistry, U. S. Department of Agriculture).*

[Grams per 100 cc.]

Data.	Unfermented—original sample of must.	Fermented.			
		Flask 1, unsown.	Flask 2, sown with 1 cc No. 8.	Flask 3, sterilized, sown with 1 cc No. 8.	Flask 4, sterilized, sown with 1 cc No. 66.
Specific gravity	1.059	1.022	1.008	1.001	1.002
Total solids	15.53	6.77	3.34	2.75	2.88
Sucrose	3.31	.90	.00	.00	.00
Reducing sugar	9.53	2.56	.81	.11	.11
Total sugar	13.01	3.51	.81	.11	.11
Acid, as sulphuric	.40	.43	.63	.41	.48
Acid, as acetic	.00	1.36	1.20	.01	.01
Alcohol		2.16	3.25	5.67	5.84
Loss of carbon dioxid		16.97	22.95	26.84	28.74

These results show clearly that yeast fermentation in flask No. 1 (unsown) was not properly developed. There remained 3.51 per cent of total sugar when the weights became fairly constant. The

explanation which seems most reasonable is that only weak yeasts occurred in this flask of juice, and at the comparatively high temperature at which it was held in the culture oven other organisms grew to such an extent as to prevent the completion of alcoholic fermentation. This is clear from the analysis, which shows that the vinegar ferment was well developed at the close of the test, as 1.36 per cent of acetic acid was found.

Flask No. 2, unsterilized, but sown with a pure-yeast culture, 1 part to 400 of juice, nearly completed the alcoholic fermentation before it was overtaken by the growth of the vinegar ferment. Flasks 3 and 4, sterilized and sown with pure-yeast cultures, show that alcoholic fermentation ran its normal course without any occurrence of acetic fermentation.

The notes on progress of fermentation and appearance of the material in each flask are even more instructive than the analytical data. The juice used in these tests was not filtered, but taken just as it ran from the press, hence the notes on formation of "head" and clearing of the liquor have value. A filtered must does not form a "head," in the practical sense of the term. Flask No. 1 was especially slow in starting, and not until forty-two hours had elapsed did it show a well-defined appearance of yeast growth, while No. 2 showed active fermentation in eighteen hours, with "head" already forming. Nos. 3 and 4 showed slight fermentation in eighteen hours; No. 2 showed a well-formed "head," liquor beginning to clear in forty-eight hours, while Nos. 3 and 4 showed active fermentation, with only a trace of light, frothy "head." The sterilized juice in flasks 3 and 4 did not take on at all the characteristic appearance of the juice that had not been heated.

After three days the liquor in flask No. 2 was a bright amber color, nearly clear, with a strong sediment at bottom, and "head" completely formed. The high temperature, together with the strong yeast sown, hastened the completion of the first, or tumultuous, fermentation in this flask, and if the liquor could have been properly drawn off at this time and finished at a lower temperature it is probable that the acetic fermentation shown by the analysis would not have developed. No. 1, after three days in the culture oven, was in strong fermentation, with "head" forming, but the liquor had a muddy and unpromising appearance. Nos. 3 and 4 were in strong fermentation, with frothy "head;" the liquor was milky and dirty in appearance. After four days' time the "head" on No. 2 was falling, but the liquor was still bright amber. No. 1 showed a fairly good "head" and sufficient deposit, but the liquor was far from clean and unsatisfactory in color. In the case of Nos. 3 and 4 the frothy "head" continued, but the liquor was not clearing.

The notes continued throughout the experiment in the same tenor and showed clearly the striking differences apparent in the character of the liquors. It is very evident, from this and numerous other tests made, that dominant fermentation with pure yeast will not destroy the troublesome organisms present, but will control the fermentation for a certain period and, as was shown in the cask experiments (Bulletin 88), it renders practical control fairly simple when the liquor is racked and handled at proper temperatures. In fact, it is possible to almost or quite eliminate the acetic and other troublesome ferments by proper attention to details and control of the temperature. When the first fermentation is accomplished, at a comparatively high temperature (75° to 80° F.), the liquor must be promptly racked and handled under control from outside contamination to prevent the development of the acetic ferments.[a]

EFFECT OF VARYING QUANTITIES OF YEAST ON RAPIDITY OF FERMENTATION.

Dry yeasts will not prove desirable in the fermentation industries because they must first be brought into active growth before they will prove efficient as "starters," and present experience indicates that their handling by ordinary methods will, without doubt, result in contamination.

It appears, therefore, that the active culture prepared by sowing a pure yeast into sterilized must is the only proper inoculating material for the fermentation of fruit juices.

In the cask work described in Bulletin 88 one pint of sterilized must fermented with a pure yeast culture was sown to 50 gallons of fresh fruit juice, just from the press, or 1 part of starter to 400 parts of must. This, in every instance, produced a strong fermentation, which appeared readily to dominate and prevail over the organisms present in the natural must. This quantity of yeast culture having proved efficient on a large scale, the same proportion was used in the laboratory experiments for the control of unsterilized must. The recommendation sent out from the German laboratory at Geisenheim directs the use of 1 part of fresh yeast culture to 250 or 300 parts of must. This practice should be varied according to conditions; so large a proportion is not necessary under the best conditions of temperature in cellars or fermentation rooms, but where the conditions are not good, 1 part to 100 should be used for safety.

An experiment bearing upon this point was conducted by the writer in the laboratory at Geisenheim, Germany, and the results are presented in fig. 3. The purpose of this test is to show the effect

[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. 71, p. 98.

of different quantities of yeast upon rapidity of fermentation. The sowings were made in a filtered and sterilized grape must of the approximate analysis shown in Table II. Three flasks containing 400 cc each of the sterilized grape juice were sown as follows: No. 1 with 1 drop on standard loop; No. 2 with 1 cc taken up in a pipette; No. 3 with 10 cc taken up in a pipette.

TABLE II.—*Partial analysis of grape must used in the experiment and the liquor after fermentation.*

[Grams per 100 cc.]

Description.	Total sugar.	Alcohol.	Acid.	Total loss of carbon dioxid.
Grape must	15.74		0.9075	
Flask No. 1		7.29	.9075	30.25
2		7.36	.9075	30.55
3		7.70	.8925	31.60

Steinberg yeast was used, the same as is denominated No. 53 in the descriptive list on page 25. The flasks were all kept in the culture oven, but the gas lamp was turned off at night. The temperature, though not maintained at the most desirable degree, was fairly constant, approximating 21° C., but with extremes of 19° C. to 25° C. The time covered by the entire experiment was twenty-four days, but the curves on the chart (fig. 3) are given for only twelve days, the later fermentation having little interest in this discussion. The diagram shows that No. 3, which received the largest amount of yeast, made a very rapid fermentation the second and third day, reaching the climax on the fourth day. This should certainly result when, as in this case, the quantity of culture used as a starter is as 1 to 40 of the must. No. 2, which received 1 to 400 parts of culture, exceeds in activity for the fourth day the heavy sowing of No. 3. This illustrates forcibly the point shown in all of this work, namely, that a small proportional inoculation with the pure culture answers every purpose, 1 part to 400 being sufficient under good conditions. The validity of this observation is borne out by flask No. 1, which received a comparatively infinitesimal sowing of 1 drop to 400 cc of must, yet, when growth fairly started on the third day, it rushed to a point comparatively high on the fourth day, and reached its climax on the fifth day.

The prompt control which can be obtained by a heavy sowing of yeast when there is pressing need of securing a dominant fermentation to guard against unfavorable conditions is most amply illustrated in this case by the prompt manner in which No. 3 started.

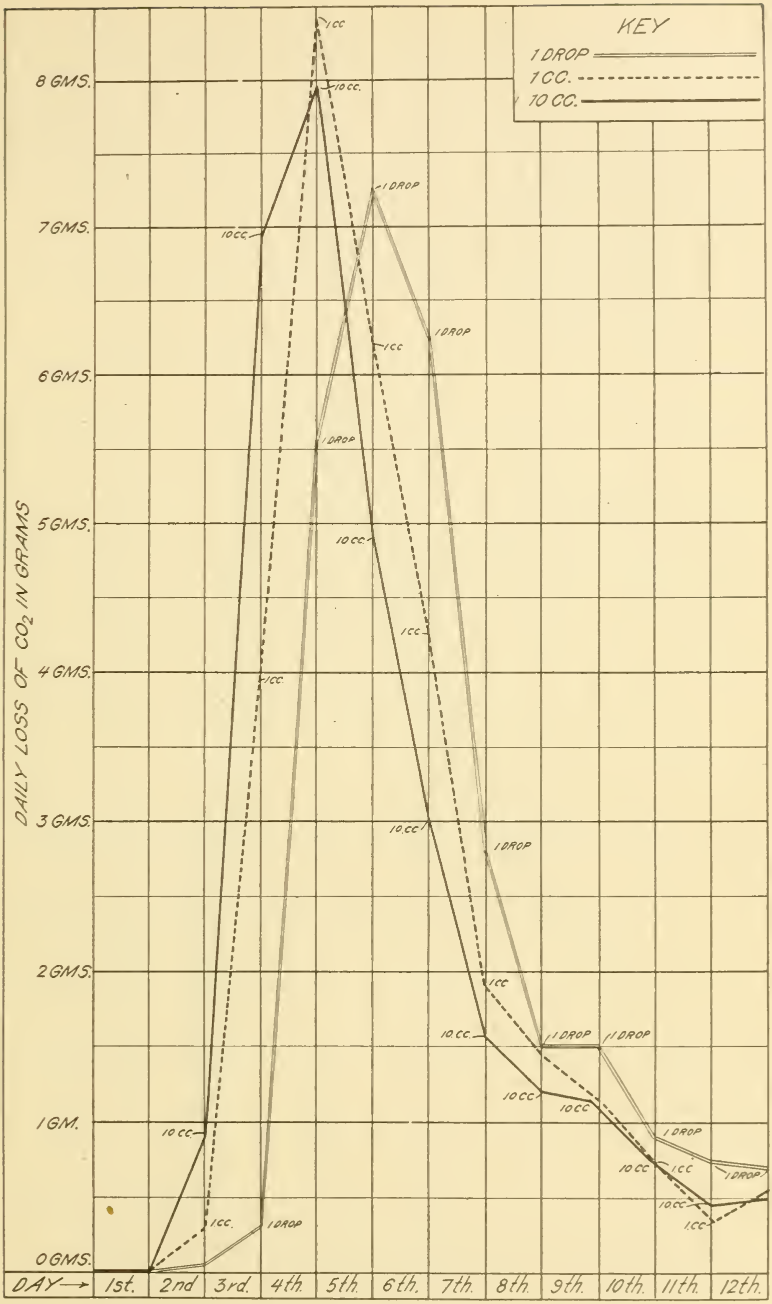

FIG. 3.—Variations in rate of fermentation produced by different quantities of yeast.

PURE YEASTS USED SEPARATELY AND IN MIXED CULTURES.

From many comparative tests of pure yeasts sown in the same must or fruit juice a set of 7 pure sowings and 1 mixed sowing is selected to illustrate the difference in activity which may be shown by pure and mixed yeasts. These tests are taken from a set of 11 flasks, which were sown at the same time under identical conditions and all kept under observation in the culture oven. The graphic record presented in fig. 4 includes 3 yeasts from French, 1 from German, and 3 from American sources, and gives the entire range of variations developed in this experiment.

The flasks contained 400 cc each of filtered and sterilized apple juice and were sown with 1 drop on a platinum loop, the mixed cultures receiving 10 drops, *i. e.*, 1 drop from each of the pure cultures used in the experiment. The yeasts used were from the following sources: Nos. 8, 37, and 40, obtained from French Normandy cider, isolated by the writer at Geisenheim, Germany; No. 53, a quite famous wine yeast, isolated at the royal Prussian laboratories at Geisenheim, Germany, from Steinberg wine; No. 66, from Yellow Newtown cider; No. 94, from Hyslop crab-apple juice; No. 100, from the same source as No. 94. The last three yeasts were isolated in the mycological laboratory at Blacksburg, Va. No. 100 is the organism known as *Saccharomyces apiculatus*, which is always present in abundance in normal fruit juices. The chemical data of the experiment are set forth in the following table:

TABLE III.—*Chemical analyses of original must and of the same after fermentation with different yeasts (chemical department, Virginia Agricultural Experiment Station).*

[Grams per 100 cc.]

Description.	Specific gravity.	Total solids.	Sucrose.	Reducing sugar.	Total sugar.	Acid as sulphuric.	Acid as acetic.	Alcohol.	Total loss of carbon dioxid.
Juice	1.066	14.60	2.65	10.36	13.15	0.51	--------	--------	--------
Yeast 8	1.003	2.78	--------	.15	.15	.55	0.02	5.05	26.30
Yeast 37	1.003	2.88	--------	.16	.16	.57	.02	5.78	25.86
Yeast 40	1.003	2.65	--------	.14	.14	.51	.02	6.01	26.81
Yeast 53	1.003	2.72	--------	.15	.15	.55	.02	5.61	26.39
Yeast 66	1.003	2.77	--------	.19	.19	.60	.02	6.36	25.51
Yeast 94	1.002	2.73	--------	.18	.18	.55	.02	6.26	26.60
Yeast 100	1.028	7.97	2.40	2.73	5.26	.60	.04	3.25	15.30
Mixture	1.003	2.69	--------	.18	.18	.60	.02	5.72	26.22

These analyses show that all of the organisms, except No. 100, gave fairly uniform results, with the exception of the data for the percentage of alcohol in the fermented liquor. It is indeed remarkable that the alcohol produced should vary more than 1 per cent, but like results have been shown repeatedly in the tests of pure yeasts. In the writer's opinion, this is not an adventitious occurrence, but a special investigation must be made before attempting a

discussion of this point. The low fermenting power of No. 100 accords entirely with what is well known as to the vital functions of this organism (*S. apiculatus*), namely, that it does not secrete the enzym

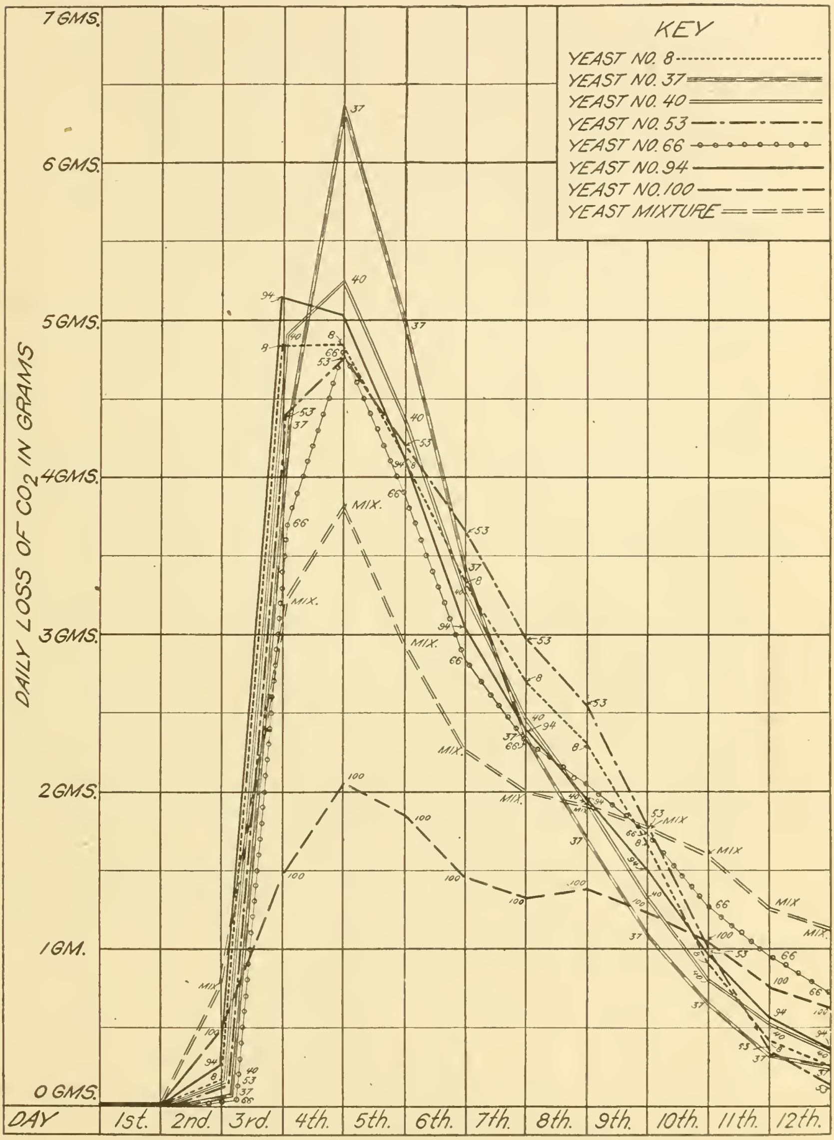

FIG. 4.—Rate of fermentation of pure and mixed yeasts.

invertase, and hence can not invert cane sugar, and that it rarely produces 3 per cent of alcohol.

The tabulated figures alone do not fully reveal the peculiarities of pure yeast races. Therefore the graphic chart (fig. 4) is used

to depict more strikingly the behavior of these organisms toward the culture substance—in this case apple juice. The weighings made at stated intervals show the destruction of sugar through the loss suffered by reason of the carbon dioxid given off. The weights were taken each twenty-four hours and the results noted on the record sheets. Fig. 4 depicts in a striking manner the daily loss of carbon dioxid by the height to which the line designating the activity of each yeast rises above the base. The chart is in fact a picture of the vital activities of each yeast organism in the test.

So far as the weighings showed no change occurred during the first twenty-four hours, but it is a fact, frequently verified by the writer by microscopic examinations, that there results a large increase in the number of yeast cells during the first day, but until the liquor is fairly saturated with the carbon dioxid gas none is given off, and therefore the destruction of sugar would not be shown by weighing. The second day all the tests showed some loss, reaching 0.75 of a gram in the case of the mixed culture. This greater activity was caused by the larger amount of yeast sown. The next most active yeast is No. 100. This is always the case when *S. apiculatus* is compared with other yeasts, and it is this precocity of development that renders it so obnoxious in the fermentation industries. In its final development No. 100 makes a poor showing compared with the other yeasts. Its maximum occurs on the fourth day at 2.05 grams, and on the same day No. 37 reaches its maximum at 6.33 grams loss of carbon dioxid, though it was one of the slowest to start.

Between these extremes lie all the other yeasts used, the mixed culture showing its climax at about half the height of No. 37. An interesting observation, which holds good in other tests, is that a mixture of several pure yeasts has not shown the same power of fermentation that many of the same yeasts show when sown alone. In this particular case, and in fact whenever *S. apiculatus* is used, this result, to a considerable extent, should probably be credited to the fact that this undesirable form is for a time dominant in the liquor to the partial exclusion of more desirable ferments.

MISCELLANEOUS ANALYSES OF FERMENTED MUSTS.

During the several years that the tests of pure yeasts and organisms associated with them have been in progress many chemical analyses of the fermented liquor have been made, and a number of these analyses are presented in Table IV for reference. Generally the yeasts do not show striking differences in the amount of alcohol produced when used in the same must. There are, however, some interesting differences, and these are brought out by the tabulated analyses. This work was undertaken to collect preliminary data on

the activity of yeasts as chemical agents, and complete check tests were not made in many cases. A considerable number of check tests and analyses were made, however, and the data herein presented represent fairly the results obtained.

The results of the analyses of must inoculated with several of the fungi associated with the yeasts are interesting. With Penicillium in one case (test 82) the destruction of 2.12 per cent of sugar is shown and only 0.74 per cent of alcohol was formed, and in the other 2.14 per cent of sugar was consumed and 1.46 per cent of alcohol formed, relatively a very high result. But the species of Aspergillus used in three long tests appeared to be incapable of forming alcohol except in one instance, where 0.28 per cent was produced by this fungus. Yet these fungi destroyed 2 per cent and over of sugar. Evidently from these results it is correct to consider these organisms disturbing agencies, especially where fermentation proceeds slowly.

The forms of Torula used from two different sources in tests Nos. 88 and 107 show decided strength as alcoholic ferments. The two cultures used were quite different as to characteristics of the cells, so much so as to indicate difference in species. The fact that this organism forms alcohol so readily renders it all the more objectionable when associated with yeasts in the fermentation industries, because during growth its secretions are likely to injure the bouquet of the product.

The three forms, or presumably species, of Mycoderma used show striking differences in their fermenting power, varying from less than one-half of 1 per cent to more than 4 per cent of alcohol. All of these inverted sucrose, No. 92 practically all of it, and consumed 11.46 per cent of sugar to form 4.15 per cent of alcohol, a poor result in that respect. In the case of this strong growing form it appears that it might become a decided competitor of yeasts, especially under conditions where fermentation progresses slowly. This fungus is also a very undesirable ferment. Dematium did not show much power to form alcohol so far as tested.

TABLE IV.—*Analyses of apple musts and resultant product after fermentation with pure cultures of yeasts and other organisms (chemical department, Virginia Agricultural Experiment Station).*

[Grams per 100 cc.]

ANALYSES OF MUSTS.

Must No.	Specific gravity.	Total solids.	Sucrose.	Reducing sugar.	Total sugar.	Acid as sulphuric.	Tannin.
1	1.057	13.32	0.53	10.40	10.96	0.49	----------
2	1.055	12.38	.27	9.10	9.38	.51	----------
3	1.055	12.64	.93	9.10	10.17	.51	----------
4	1.066	14.60	2.65	10.36	13.15	0.51	0.018
5	1.060	14.05	3.81	9.16	13.28	.45	.0387
6	1.060	15.02	2.37	11.22	13.72	.46	.018

ANALYSES OF FERMENTED PRODUCTS.

Product of Must No. 1.

Test No.	Date of fermentation.	Serial No. of yeasts.	Specific gravity after fermentation.	Loss of CO_2 during fermentation.	Total solids.	Sucrose.	Reducing sugar.	Total sugar.	Alcohol.	Total acid as sulphuric.	Volatile acid as acetic.	Tannin.	Duration of test.	Mean temperature during fermentation.	Source of yeast.
	1901.												*Hours.*	° *C.*	
1	July 11	53	1.004	21.37	2.59	--------	0.12	0.12	5.17	0.56	--------	--------	382	25.86	Steinberg wine (German).
2	do	55	1.003	21.42	2.23	--------	.16	.16	5.02	.54	--------	--------	333	25.57	Winningen wine (German).
3	do	51	1.004	19.74	2.77	--------	.16	.16	4.87	.55	--------	--------	333	25.57	Rudesheimerberg wine (German).
23	Aug. 17	45	1.006	19.57	2.73	--------	.18	.18	4.68	.52	--------	--------	262	25.45	Assmanshausen wine (German).
24	do	47	1.004	19.60	2.32	--------	.19	.19	4.91	.55	--------	--------	262	25.45	Champagne Ay wine (German).
25	do	48	1.005	20.07	2.78	--------	.15	.15	4.68	.57	--------	--------	334	24.85	Laureiro wine (German).
5	July 16	7	1.003	21.32	2.30	--------	.17	.17	5.11	.55	--------	--------	263	26.51	French cider.
6	do	8	1.004	21.21	2.70	--------	.19	.19	4.90	.52	--------	--------	239	26.41	Do.
7	do	10	1.004	20.82	2.70	--------	.18	.18	4.87	.58	--------	--------	239	26.3	Do.
8	July 20	9	1.004	20.98	2.86	--------	.16	.16	5.26	.56	--------	--------	256	27.66	Do.
9	July 23	12	1.005	20.86	2.70	--------	.26	.26	5.36	.53	--------	--------	256	27.22	Do.
10	July 20	13	1.005	20.97	2.60	--------	.17	.17	4.93	.50	--------	--------	280	27.37	Do.
12	July 29	18	1.005	19.52	2.37	--------	.17	.17	4.42	.54	--------	--------	255	24.73	Do.
13	do	19	1.006	19.07	2.80	--------	.19	.19	4.38	.56	--------	--------	257	24.73	Do.
14	do	30	1.004	19.33	3.00	--------	.19	.19	4.84	.52	--------	--------	281	24.75	Do.
15	Aug. 1	33	1.006	19.00	2.39	--------	.19	.19	4.13	.55	--------	--------	305	24.34	Do.
16	do	35	1.007	19.24	2.97	--------	.22	.22	3.95	.54	--------	--------	305	24.27	Do.
17	Aug. 6	37	1.006	18.56	2.86	--------	.22	.22	4.17	.62	--------	--------	312	24.41	Do.
18	do	39	1.006	18.68	2.46	--------	.28	.28	4.13	.58	--------	--------	312	24.36	Do.
19	Aug. 9	40	1.004	19.80	2.76	--------	.18	.18	4.15	.59	--------	--------	307	25.00	Do.

20	do	43	1.008	19.55	2.45	--------	.19	.19	4.86	.58	--------	--------	307	25.00	Do.
21	Aug. 10	16	1.005	19.81	2.86	--------	.18	.18	4.28	.54	--------	--------	304	25.00	Do.
22	do	44	1.006	19.61	2.57	--------	.20	.20	4.18	.54	--------	--------	304	25.00	Do.
29	Aug. 24	71	1.005	18.81	2.69	--------	.17	.17	4.45	.54	--------	--------	305	23.48	Do.
30	do	70	1.006	19.01	2.77	--------	.16	.16	4.44	.61	--------	--------	305	23.39	Do.
33	Aug. 29	59	1.006	19.10	2.29	--------	.16	.16	4.16	.53	--------	--------	358	23.31	Do.
27	Aug. 23	64	1.005	19.16	2.69	--------	.15	.15	4.46	.61	--------	--------	281	23.78	American cider.
									Product of Must No. 2.						
37	Sept. 7	57	1.005	19.19	2.94	--------	0.19	0.19	4.32	0.58	--------	--------	305	22.01	American wine.
38	do	60	1.005	19.48	2.77	--------	.17	.17	4.10	.48	--------	--------	305	22.01	Do.
									Product of Must No. 3.						
	1902.														
63	Sept. 15	59	1.005	24.71	2.90	--------	0.13	0.13	5.42	0.67	--------	0.020	102	22.68	French Bordeaux.
64	do	61	1.005	21.10	2.88	--------	.21	.21	5.42	.71	--------	.021	354	22.85	French champagne.
65	do	74	1.003	24.71	2.81	--------	.06	.06	5.49	.70	--------	.019	102	22.74	French cider.
67	do	73	1.005	23.68	3.01	--------	.18	.18	5.19	.73	--------	.020	354	22.85	French sauternes.
									Product of Must No. 4.						
68	Oct. 24	8	1.003	26.30	2.78	--------	0.15	0.15	5.02	0.55	0.02	0.016	427	22.47	French cider.
69	do	9	1.003	25.79	2.91	--------	.18	.18	4.97	.57	.02	.015	498	22.62	Do.
70	do	37	1.003	25.86	2.88	--------	.16	.16	5.78	.57	.02	.017	402	22.40	Do.
71	do	40	1.003	26.81	2.65	--------	.14	.14	6.01	.51	.02	.016	570	22.68	Do.
72	do	53	1.003	26.39	2.72	--------	.15	.15	5.61	.55	.02	.017	402	22.4	German wine, Steinberg.
73	do	91	1.002	26.60	2.73	--------	.18	.18	6.26	.55	.02	.016	497	22.61	American crab apple.
74	do	93	1.005	26.31	3.12	--------	.12	.12	5.95	.48	.04	.017	1,097	22.77	Do.
75	do	97	1.004	26.35	3.05	--------	.12	.12	6.01	.47	.01	.018	1,097	22.77	Do.
76	do	66	1.003	25.51	2.77	--------	.19	.19	6.36	.60	.02	.015	401	22.4	American cider.
77	do	100	1.028	15.30	7.97	2.40	2.73	5.26	3.25	.60	.04	.015	568	22.72	American crab apple.
78	do	(a)	1.003	26.22	2.69	--------	.18	.18	5.72	.60	.02	.016	592	22.72	

a Preceding 10 yeasts mixed.

TABLE IV.—*Analyses of apple musts and resultant product after fermentation with pure cultures of yeasts and other organisms (chemical department, Virginia Agricultural Experiment Station)*—Continued.

ANALYSES OF FERMENTED PRODUCTS—Continued.

Test No.	Date of fermentation.	Serial No. of yeasts.	Specific gravity after fermentation.	Loss of CO_2 during fermentation.	Total solids.	Sucrose.	Reducing sugar.	Total sugar.	Alcohol.	Total acid as sulphuric.	Volatile acid as acetic.	Tannin.	Duration of test.	Mean temperature during fermentation.	Source of yeast.
Product of Must No. 5.															
	1903.												*Hours.*	° *C.*	
95	Feb. 14	106	1.005	23.20	2.50	--------	Trace.	Trace.	5.84	0.53	--------	0.0317	665	21.49	American cider.
96	do	101	1.004	22.69	2.45	--------	Trace.	Trace.	5.28	.54	--------	.0369	665	21.49	American scuppernong wine.
97	do	103	1.004	22.93	2.54	--------	Trace.	Trace.	5.18	.49	--------	.0388	665	21.49	American cider.
104	Mar. 10	124	1.001	23.50	2.40	--------	0.008	0.008	5.83	.53	0.005	.001	857	22.3	American Ives grapes.
106	do	130	1.001	22.62	2.43	--------	.10	.10	6.04	.50	--------	.014	905	22.38	American wine.
125	Apr. 22	86	1.003	23.43	2.71	--------	.08	.08	5.59	.47	.005	.020	550	22.89	Do.
126	do	88	1.003	21.73	2.77	0.012	.028	.041	4.99	.47	.0054	.025	622	22.74	American Yellow Newtown apples.
127	do	89	1.005	21.58	3.07	.042	.086	.130	4.73	.578	.0052	.023	718	22.89	American Norton grapes.
Product of Must No. 6.															
133	May 18	91	1.006	23.80	2.54	--------	0.28	0.28	6.86	0.56	--------	0.017	455	22.65	American mixed apples.
134	do	93	1.005	23.91	1.87	--------	.26	.26	7.17	.54	--------	.016	526	22.73	American crab apples.
135	do	94	1.003	25.45	1.91	--------	.15	.15	6.76	.54	--------	.016	383	22.85	Do.
136	do	96	1.009	24.56	2.06	--------	.28	.28	6.44	.56	--------	.018	503	22.62	American Norton grapes.
Product of Must No. 4 with Mal-Ferments.															
	1902.														
82	Dec. 13	145	1.067	4.47	13.77	0.21	10.81	11.03	0.74	0.51	0.03	0.015	2,226	22.00	Penicillium.
84	do	144	1.056	4.55	18.35	--------	11.01	11.01	1.46	.51	--------	.018	1,986	22.25	Do.
85	Dec. 15	143	1.064	2.10	15.01	.15	12.01	12.17	.28	.36	--------	.018	1,938	22.00	Aspergillus.
86	do	142	1.063	3.34	14.50	--------	11.32	11.32	--------	.31	.01	.018	2,202	22.00	Do.
87	do	141	1.063	2.63	14.81	.56	11.09	11.68	--------	.46	--------	.014	2,178	--------	Do.
91	Feb. 10	147	1.060	4.36	5.60	2.12	.41	2.64	4.37	.44	--------	.012	1,410	22.75	Torula.
88	do	110	1.020	20.65	15.01	1.33	10.65	12.05	.42	.53	.06	.012	2,010	22.00	Mycoderma.
	1903.														
90	do	135	1.063	2.23	14.80	1.00	10.00	11.05	.57	.40	--------	.010	1,367	22.00	Dematium.
91-*a*	do	153	1.045	10.09	11.17	1.00	7.18	8.23	1.59	.70	.25	.011	1,961	22.00	Mycoderma.
92	do	121	1.022	21.37	7.30	.01	1.69	1.69	4.15	.53	--------	.007	1,679	22.50	Do.
107	Mar. 13	99	1.027	10.5	7.80	1.50	3.68	5.26	3.18	.52	.10	--------	689	21.44	Torula.

DESCRIPTIVE LIST OF YEASTS AND OTHER ORGANISMS.[a]

[The more valuable yeasts here mentioned are furnished in small quantities, with instructions for their use, to persons desirous of experimenting with them.]

Laboratory No. 8. A yeast isolated by the writer from French cider obtained at St. Ouen de Thouberville, France.

Laboratory Nos. 9 to 44, inclusive, are yeasts isolated from the same source as the above.

Laboratory No. 45. A German wine yeast purchased from the Royal Pomological Institute, at Geisenheim. This yeast is one of the regular collection used for wine making in the Rheingau and is known as "Assmanshausen." It is especially recommended for red wines.

Laboratory No. 46. A yeast purchased at Geisenheim, as above, and called "Bordeaux" yeast. It was derived from a French wine and is used by the Germans for the fermentation of red wine.

Laboratory No. 47. A yeast purchased from the same source as the above, and known as "Champagne Ay." It is used for the finishing of champagne wines and was derived from a French source.

Laboratory No. 48. A German yeast from the laboratory at Geisenheim, known as "Laureiro." It was derived from a German source and is used for sweet wines.

Laboratory No. 49. A yeast obtained from the Geisenheim laboratory, known as "Liebfrauenmilch," and used for white wines. It was obtained from the German wine of like name.

Laboratory No. 50. A German yeast obtained from Geisenheim, known as "Piesport." It is used for making apple wines.

Laboratory No. 51. A German yeast from the laboratory at Geisenheim, known as "Rudesheimer-Berg." This yeast was obtained from the Rudesheimer wine and is used for white wines and for apple wine.

Laboratory No. 52. A German yeast obtained from the laboratory at Geisenheim, known as "Schloss-Vollrads." It is also used for white wines and apple wines.

Laboratory No. 53. A German yeast obtained from the Geisenheim Laboratory and known as "Steinberg." It is one of the best known yeasts of the Geisenheim Laboratory and is used for white wines.

Laboratory No. 54. A German yeast obtained from the Geisenheim Laboratory and known as "Ungarn" (Menes) and is used for sweet wines.

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[a] Where not otherwise specified, the organisms mentioned were isolated by author.

Laboratory No. 55. A German yeast from the Geisenheim Laboratory, known as "Winningen," and is used for apple wine.

Laboratory No. 56. A German yeast obtained from the Geisenheim Laboratory and known as "Zeltingen;" it is used for apple wines.

Laboratory No. 57. A yeast separated from wine lees marked "Bois de Terrior," from Alsace, Germany, separated at Blacksburg, Va.

Laboratory No. 58. A yeast obtained from the same material as above.

Laboratory No. 59. A yeast separated from a culture received from Institute La Claire. This culture was labeled "Bordeaux," but proved, on examination, to contain several types.

Laboratory No. 60. A yeast separated from wine lees, marked "Borderies," from Alsace, Germany.

Laboratory No. 61. A yeast separated from a culture from the Institute La Claire, labeled "Champagne," separated at Blacksburg, Va.

Laboratory No. 62. A yeast separated from a "Sparkling draft cider" obtained from Rochester, N. Y.

Laboratory No. 63. A yeast obtained from a cider received from Huntington, Long Island, N. Y. This cider was of good quality, and was made from Yellow Newtown (*Yellow Newtown Pippin*) apples.

Laboratory No. 64. A yeast obtained from a cider made from Lady apples; same locality as No. 63.

Laboratory No. 65. A yeast separated from a bottle of Pippin cider made at Huntington, Long Island, N. Y., which had been in glass ten years; same source as the above. This yeast was characterized by its phenomenal slowness of action, and no amount of stimulation under good conditions sufficed to restore it to normal activity, yet it eventually fermented the must practically to dryness.

Laboratory No. 66. A yeast obtained from Pippin cider, one year in bottle. This yeast proved to be one of the best American yeasts isolated. From same locality as No. 65.

Laboratory No. 67. A yeast isolated from wine made from Clinton grapes. The wine was manufactured at Lawrenceville, Va.

Laboratory No. 68. A yeast isolated from Concord wine, from the same source as No. 67.

Laboratory No. 69. A yeast isolated from the same source as No. 67.

Laboratory No. 70. A yeast isolated at Blacksburg, Va., from lees of wine marked "Fine Bois" obtained from Alsace, Germany.

Laboratory No. 71. A yeast isolated from the same source as the previous, and characterized by the fact that the cells remain in colonies, so that the liquor is not cloudy during fermentation.

Laboratory No. 72. A yeast isolated from wine lees obtained from Alsace, Germany, and labeled "Fine Champagne."

Laboratory No. 73. A yeast isolated from a culture from the Institute La Claire, France. This was labeled "Sauternes," and has proven to have good qualities for making sparkling cider.

Laboratory No. 74. A yeast isolated from a culture received from the same source as above. It was labeled "Valleé d'Auge." This yeast makes a specially good dry cider.

Laboratory No. 76. A culture of *S. ludwigii*, received from the U. S. Department of Agriculture.

Laboratory No. 86. A yeast isolated from mixed apple juice at Blacksburg, Va.

Laboratory No. 87. A yeast isolated from Yellow Newtown (*Albemarle Pippin*) apples, Blacksburg, Va.

Laboratory No. 88. A yeast isolated from the same fruit as No. 87.

Laboratory No. 89. A yeast isolated from Norton Virginia grape, Blacksburg, Va.

Laboratory No. 90. A yeast isolated from Norton Virginia grape, same source as No. 89.

Laboratory No. 91. A yeast separated from apple juice taken from the cider mill at Blacksburg, Va.

Laboratory No. 92. A yeast isolated from the same source as No. 91.

Laboratory No. 93. A yeast isolated from the fruit of Hyslop crab apple. The colonies of this yeast grow in coagulated masses.

Laboratory No. 94. A yeast isolated from the same source as No. 93, the colonies of which do not grow in coagulated masses.

Laboratory No. 96. A yeast separated from Norton Virginia grape, the colonies of which grow in coagulated masses.

Laboratory No. 97. A yeast isolated from Soulard crab apple at Blacksburg, Va. The colonies grow in coagulated masses.

Laboratory No. 99. A Torula from a specimen of Lankford apple, Blacksburg, Va.

Laboratory No. 100. *Saccharomyces apiculatus* isolated from Hyslop crab apple, Blacksburg, Va.

Laboratory No. 101. A yeast isolated from Scuppernong wine made in Virginia.

Laboratory No. 102. A yeast isolated from the same source as No. 101.

Laboratory No. 103. A yeast isolated from mixed apples, Blacksburg, Va.

Laboratory No. 106. A yeast isolated from a bottle of finished cider, made at Blacksburg, Va.

Laboratory No. 110. Torula separated from Soulard crab apple, Blacksburg, Va.

Laboratory No. 121. Mycoderma separated from Ives grapes, Blacksburg, Va.

Laboratory No. 124. A yeast from Ives grapes, Blacksburg, Va.

Laboratory No. 130. A yeast separated from Hock wine, from Charlottesville, Va.

Laboratory No. 135. A yeast from Quaker Beauty crab apples, Blacksburg, Va.

Laboratory No. 136. A beer yeast named "Joliet No. 1," from Chicago, Ill. A bottom yeast.

Laboratory No. 137. A beer yeast known as "Indianapolis No. 2," obtained from the same firm as No. 136. A bottom yeast.

Laboratory No. 138. An ale yeast from the same firm as No. 136, but isolated at Copenhagen, Denmark, by Hansen. A top yeast.

Laboratory No. 139. An ale yeast from the same firm as No. 136, but obtained at Sunderland, England. A top yeast.

Laboratory No. 141. Aspergillus separated from apple must.

Laboratory No. 142. Aspergillus from same source as No. 141; appears to be different species.

Laboratory No. 143. Aspergillus separated from Hyslop crab apple, Blacksburg, Va.

Laboratory No. 144. Penicillium separated from mixed apples, Blacksburg, Va.

Laboratory No. 145. Penicillium from Soulard crab apple, Blacksburg, Va.

Laboratory No. 147. *Dematium pullulans* separated from a prepared sugar solution, which accidentally became infected in the laboratory.

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